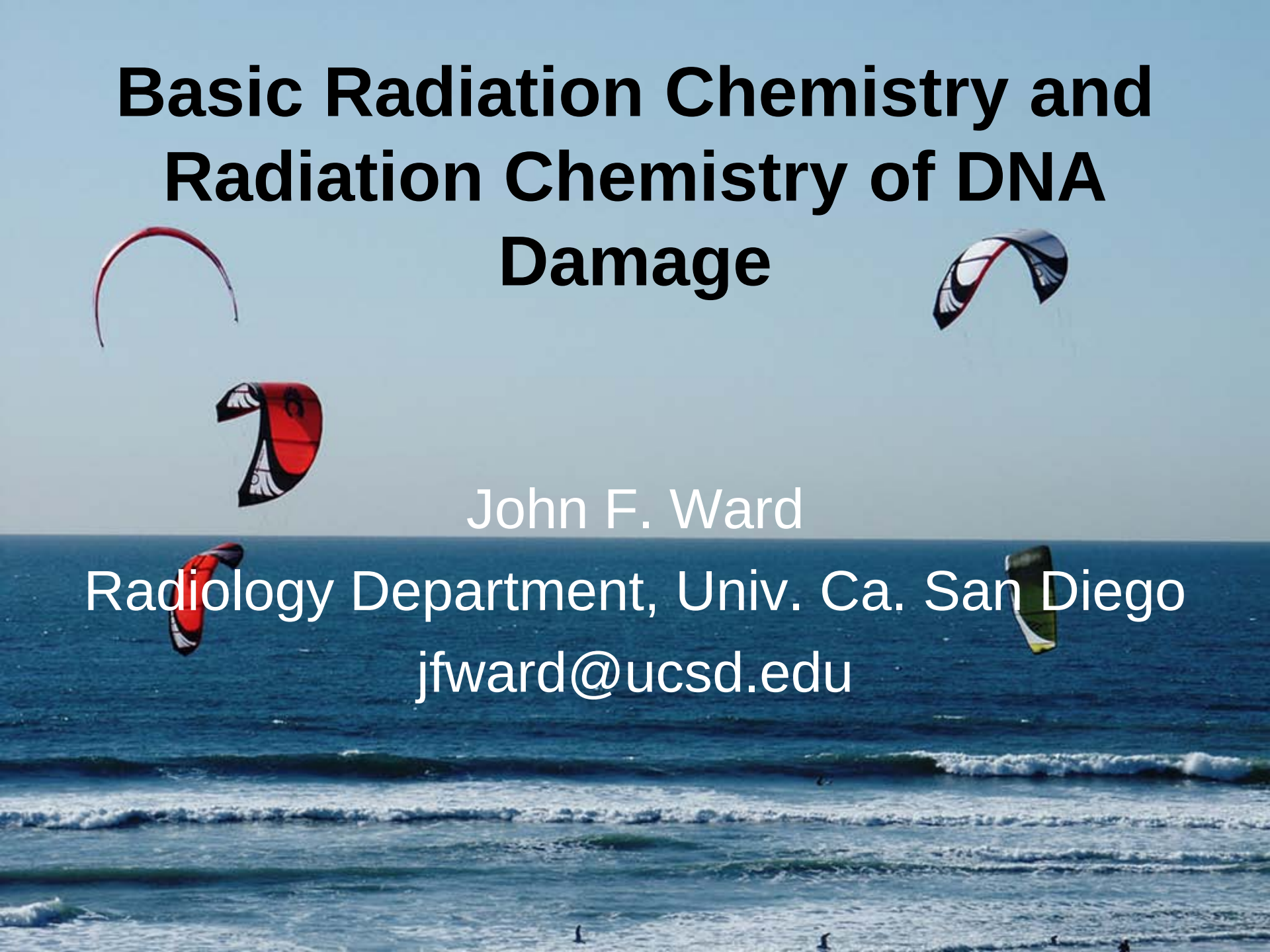
Basic Radiation Chemistry and Radiation Chemistry of DNA Damage



John F. Ward

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Ernest Rutherford

“In science there is only physics; all the rest is stamp collecting”

Responsible for naming alpha, beta and gamma ‘particles’.

Nobel Prize for Chemistry in 1908, first to split the atom in 1917.

Types of Ionizing Radiation

	Identity	Mass
α particles	$^{4}He^{++}$	4
ß particles	e^{-}	0.00054
γ rays	photon	
Protons	$^{1}H^{+}$	1
X-rays	photon	
Heavy Ions		>1

***>99% of the energy deposited by all these types of radiation is by interactions of secondary electrons with matter*.**

Time Scale (in seconds) of Radiation Action

10^{-15} Radiation energy is deposited, and ionizations occur

10^{-12}

10^{-9} Primary Radical reactions

10^{-6} Oxygen reacts with radicals

10^{-3} Permanent products are formed

10^{0} Cell begins to respond biochemically

10^{3} Enzymatic repair
Mutation/transformation occur

10^{6} Cell death scored

Cancer appearance

Ways of Determining Radiation Damage to Cells

- *In vitro* Model compounds irradiated in solution or alone
- *In vivo* Irradiation of living cells
- *In silico* Computer Models of “biophysical” processes

Biophysical models

Do not take into account important variables, e.g.

Effects of oxygen

Presence of sensitizers or protectors

Identities of radiation products

Amounts of each type of damage

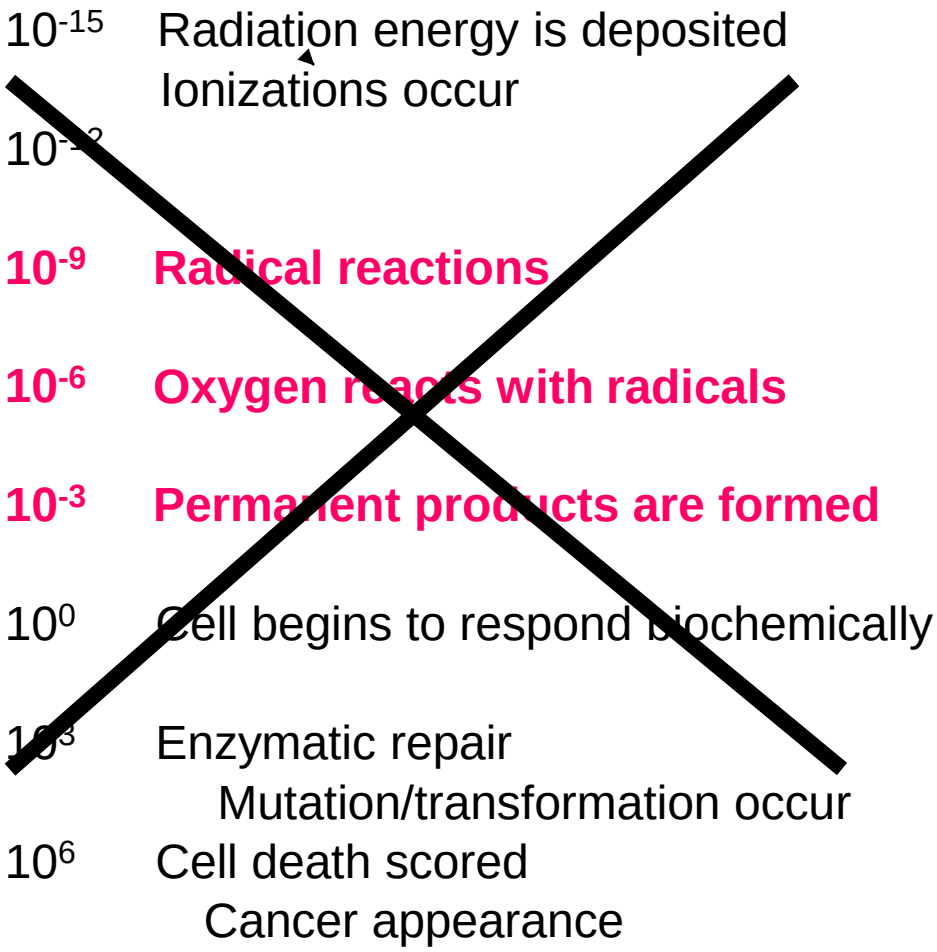
Response of cells to the damage

Effects of cell cycle

Genetic differences between cells

Conclusion: These models are very limited in their ability to predict any effects of variables.

Time Scale (in seconds) of Radiation Action



10^{-15} Radiation energy is deposited
Ionizations occur

10^{-12}

10^{-9} Radical reactions

10^{-6} Oxygen reacts with radicals

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Mutation/transformation occur

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Cancer appearance

Sidney Brenner, *Science*, July 17, 1992

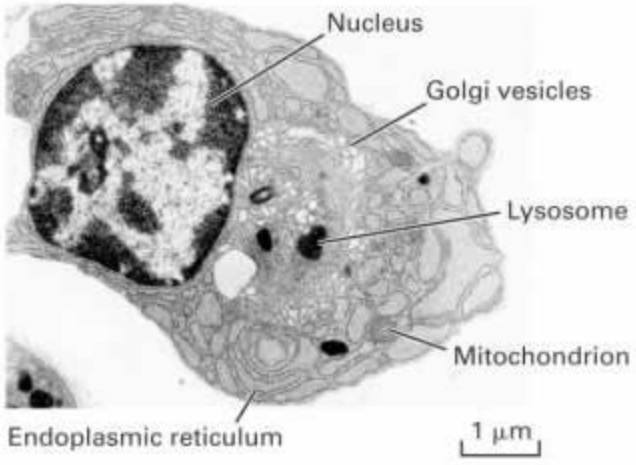
Those who prefer the airy realm of theory to the area of decisive experiment aren't necessarily doing so by choice:

I always say it's important to distinguish between chastity and impotence.

Contents of Typical Cell

From B. Alberts, Molecular Biology of a Cell, 4th Edition, Garland Press.

Cell Size 4 x 10^{-9} cm^{-3}, Density 1.15 g/cm^3 – Mass 4.6 x 10^{-9} g



	%
ter	70
rganic Ions	1
all Metabolites	3
teins	18
A	1.1
DNA	0.25
Phospholipids	3
Other lipids	2
Polysaccharides	2

Water Radiolysis

H_2O radiation $\rightarrow$ $\mathbf{H_2O^+}$ + $\mathbf{e^-}$

(ionization) *(unstable)*

$\mathbf{H_2O^+}$ + H_2O $\rightarrow$ H_3O^+ + $\mathbf{^{\bullet}OH}$

$\mathbf{e^-}$ + bulk water $\rightarrow$ $\mathbf{e\,(H_2O)_6^-}$

(hydrated electron)

Radical Damage to Intracellular Molecule (RH)

$RH \xrightarrow{\text{ionization}} RH^+$ (1)

$H_2O \xrightarrow{\text{ionization}} H_2O^+ \rightarrow OH^\bullet$ (2)

$RH^+ \rightarrow R^\bullet + H^+$ (3)

$RH + OH^\bullet \rightarrow R^\bullet + H_2O$ (4)

$R^\bullet + O_2 \rightarrow RO_2^\bullet$ (5)

$R^\bullet + GSH \rightarrow RH + GS^\bullet$ (6)

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1 and 2 occur within 10^{-12} s.

3 and 4 in 10^{-9} s.

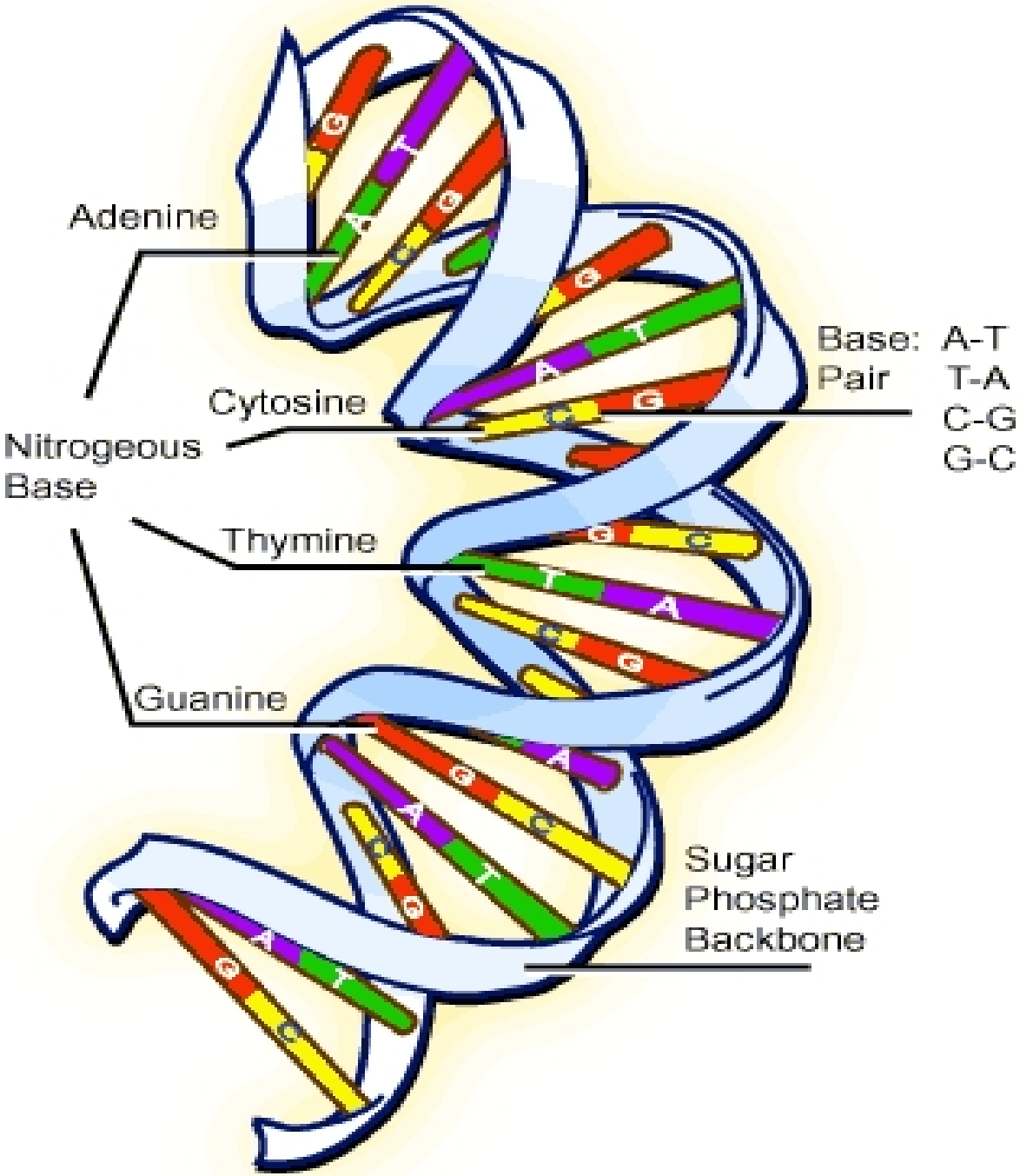
5 and 6 in 10^{-5} s.

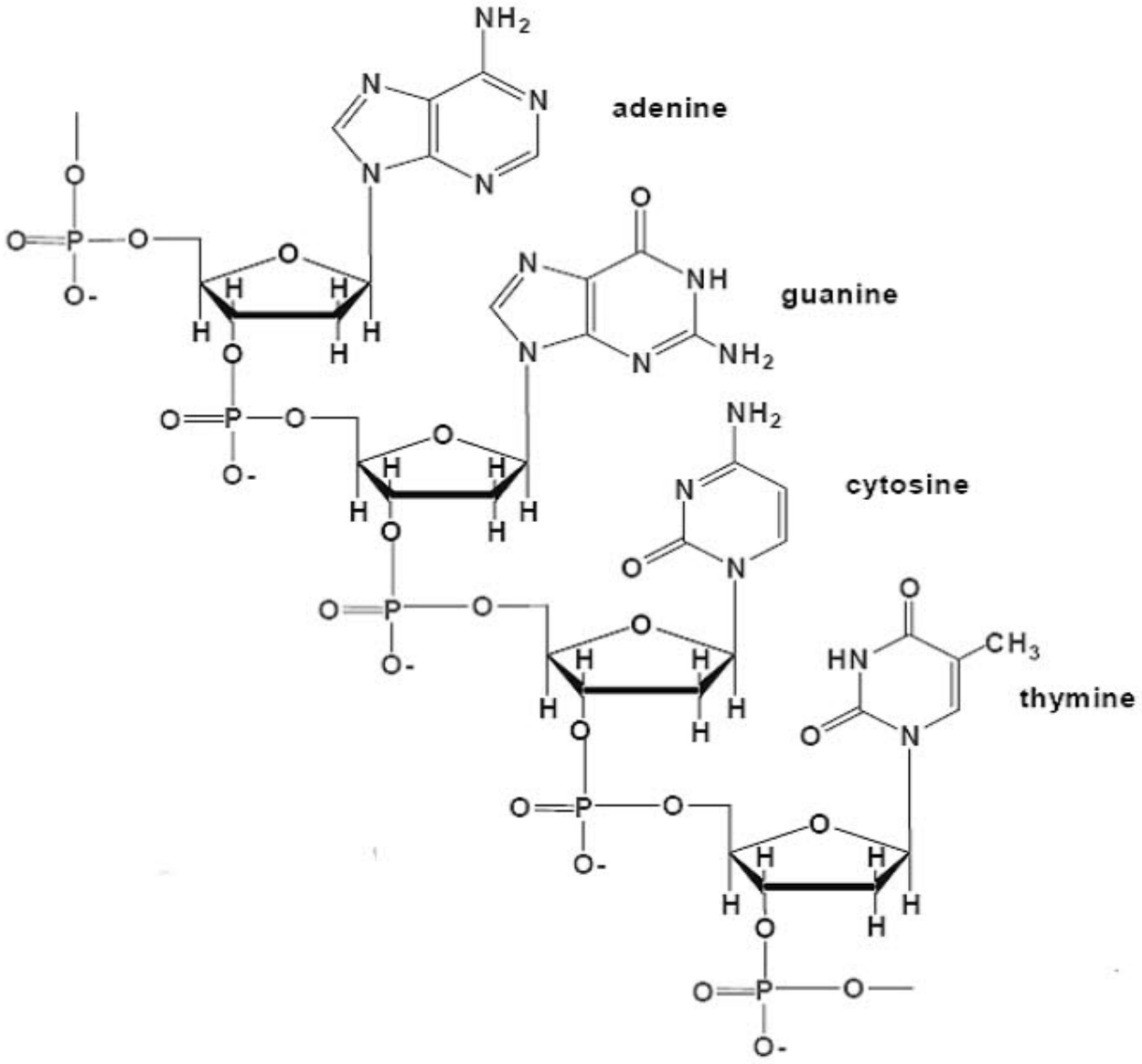
Techniques used in Radiation Chemistry.

Pulse radiolysis

Electron Spin Resonance

Quantitative analysis of products


Adenine
Cytosine
Nitrogeous Base
Thymine
Guanine
Base: A-T
Pair T-A
C-G
G-C
Sugar Phosphate Backbone


adenine
guanine
cytosine
thymine

Typical Rate Constants

a. OH radicals

		Liters per mole per second
DNA	bases	5×10^9
	deoxyribose	1.2×10^9
Amino acids –	glycine	1.7×10^7
	tryptophan	1.3×10^{10}
Ethanol		1.3×10^9
Dimethyl sulfoxide		6.5×10^9

Typical Rate Constants

b. Hydrated electrons

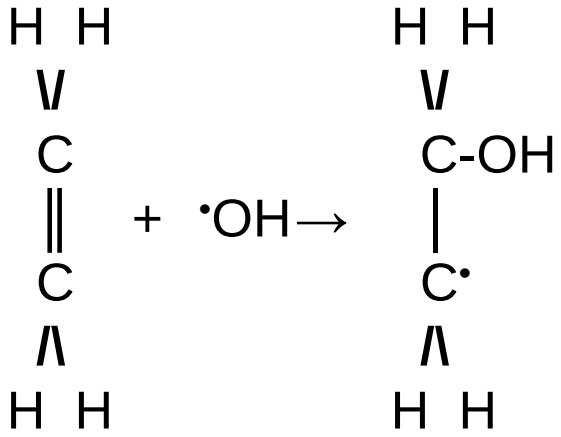
	Liters per mole per second
Oxygen	1.3×10^{10}
DNA bases	5×10^{9}
deoxyribose	1×10^{7}
Amino acids - glycine	1×10^{7}
tryptophan	3.2×10^{8}
cystine	1.1×10^{10}

Reactions of OH Radicals

1. Hydrogen atom abstraction

$$RH + {}^{\bullet}OH \rightarrow R^{\bullet} + H_2O$$

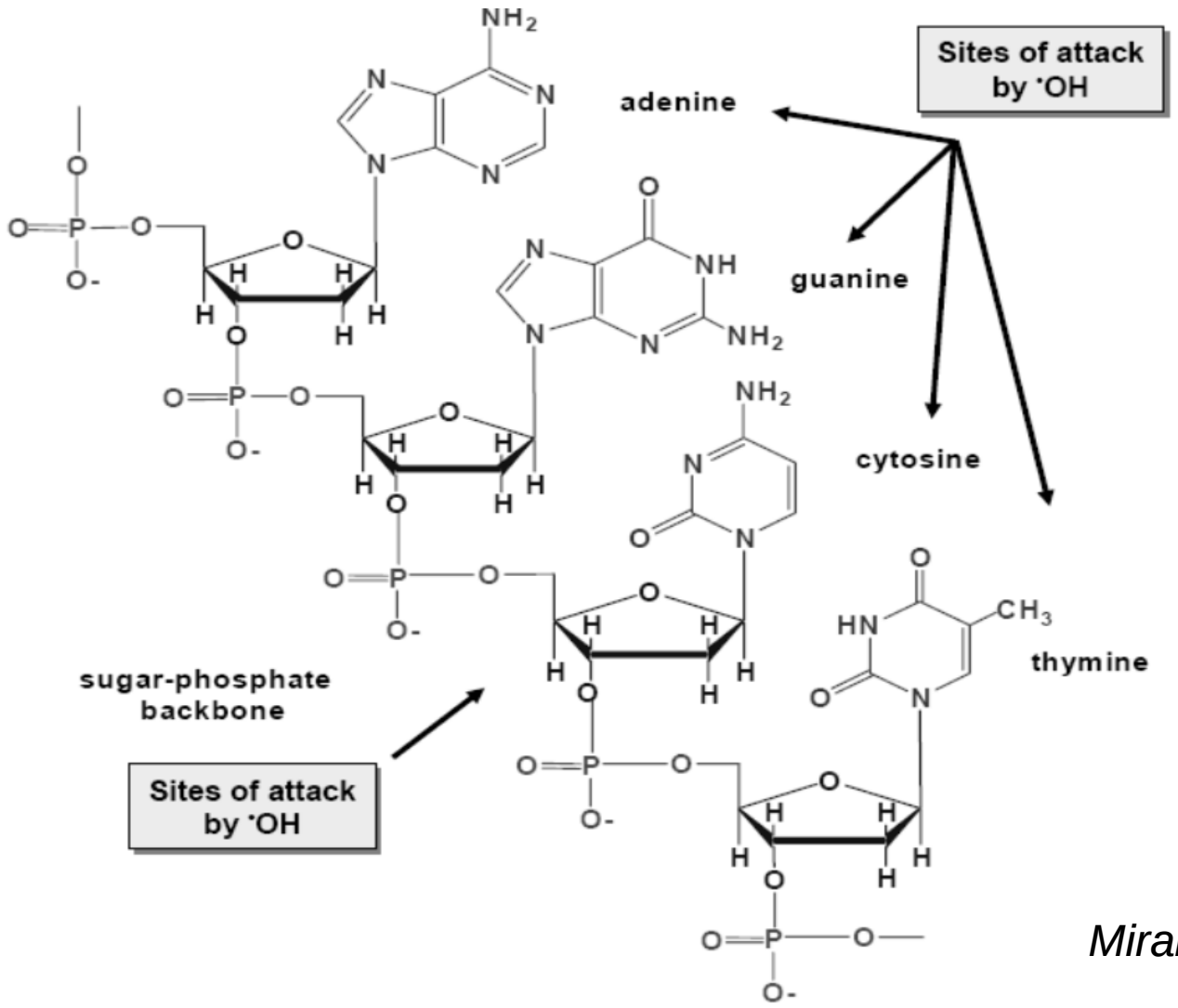
2. Addition to a double bond



3. Oxidation

$$M^{+} + {}^{\bullet}OH \rightarrow M^{++} + OH^{-}$$

OH radical attack on DNA



Miral Dizdaroglu

DNA base alterations produced by OH Radicals

Fuciarelli et al., Int. J. Radiat. Biol 58; 397.

Base Alteration	% of total base damage
8-hydroxyguanine	43
8-hydroxyadenine	7
Formamidopyrimidine-adenine	3
Formamidopyrimidine-guanine	6
Thymine glycol	27
Cytosine glycol	14

Also double lesions *Box et al., Free Radicals Biol. Med. 31; 856.*

Characteristics of Single Strand Breaks (SSB)

- Are induced by both direct ionization (35%) and OH radicals (65%). *Roots and Okada Int. J. Radiat. Biol. 21 329.*
- 15 % of OH radicals reacting with DNA cause SSB. *Scholes et al. J. Molec. Biol. 2 379.*
- 30% of directly ionizing events cause SSB. *Raskasovskiy et al Radiat Res. 153 436.*
- A base is released at the site of each SSB. *Ward and Kuo 66 485.* The termini of SSB are 5' phosphates and 3' phosphoglycolates (35%) and 3' phosphates (65%). *Henner et al. J.Biol. Chem. 258 713.*
- 70% of SSB are overt breaks, 30% are alkali labile sites. *LaFleur et al. Int. J. Radiat.Biol. 30 223.*
- Alkali labile (abasic) sites are not the same as acid induced abasic sites. *LaFleur et al. Int. J. Radiat.Biol. 35 241.*
- Base damage (BD) produced by OH radicals occurs 2.6 times more frequently than single strand breaks (SSB). *Milligan et al. Radiat. Res. 146 436.*

Direct Ionization of DNA

Initial events (observed at 4ºK) *e.g. Debije and Bernhard, J. Phys. Chem. B 104, 7845.*

Electron loss leads to guanine cation radicals and deoxyribose radicals.

Electron gain leads to pyrimidine anion radicals.

Products, (after warming and dissolution):

Bases, same types of alterations as those from •OH radical attack. *Swarts et al. Radiat. Res. 145, 304.*

30% of directly ionizing events cause SSB. *Raskasovskiy et al Radiat Res. 153, 436.*

What has been learned from *in vitro* radiation chemistry?

1. Structures of altered bases.

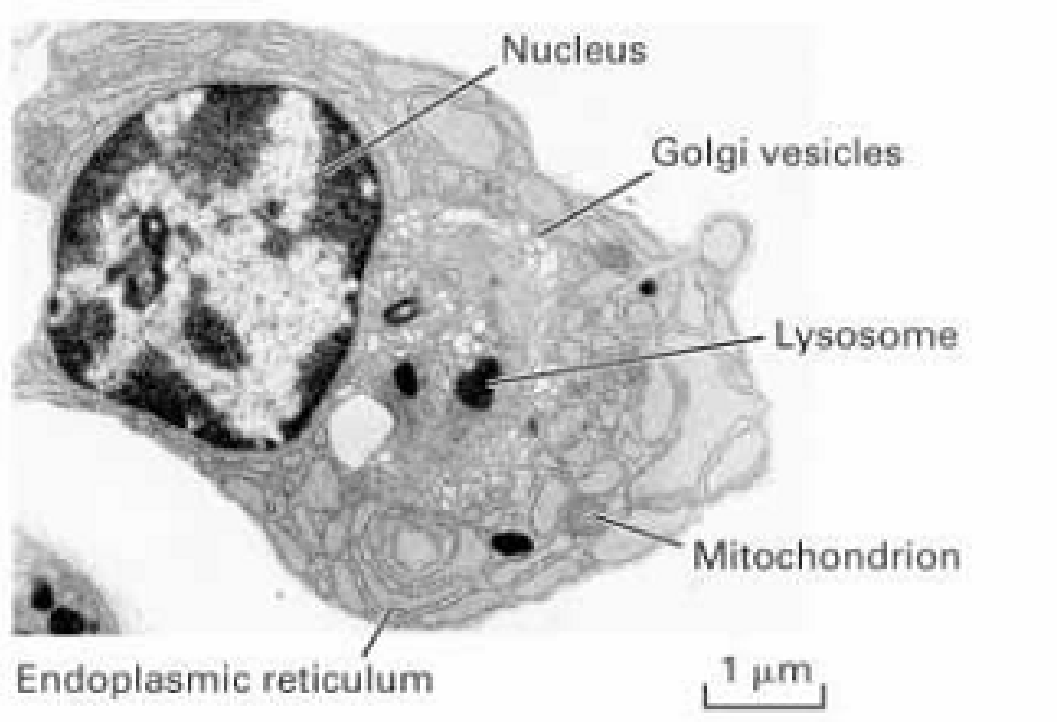
2. Strand break end-groups.

3. “Direct” and “Indirect” mechanisms cause the same types of damage.

4. Relative yields of these damages.

5. Reactions in which oxygen or radiation modifiers might act.

In vivo radiation chemistry



Ionizations in a Typical Cell

Cell Size 4×10^{-9} cm^{-3}, Density 1.15 g/cm^3 – Mass 4.64×10^{-9} g

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	%	picograms	Ionizations/Gray
Water	70	3250	1,000,000
Inorganic Ions	1	46	
Small Metabolites	3	139	
Proteins	18	835	260,000
RNA	1.1	51	16,000
DNA	0.25	12	3,600
Phospholipids	3	35	10,800
Other lipids	2	23	7,200
Polysaccharides	2	23	7,200

Could Damage to a Protein be Significant?

By definition: 1 Gray = I Joule per kgm. ≡ 6.25×10^{15} eV per gm

~ 20 eV are needed to cause an ionization.
Therefore 1 Gy causes **3.1×10^{14}** ionizations per gram

Assume **Protein** X of Mol. Wt. 100,000 present in an amount of 20,000 molecules per cell.

Mass of protein per cell = $10^5 \times 2 \times 10^4$ Daltons
= $[10^5 \times 2 \times 10^4 / 6 \times 10^{23}] \times 10^{15}$ femtograms
= **3.3 fgm**

Number of ionizations in 3.3 femtograms would be $3.1 \times 10^{14} \times 3.3 \times 10^{-15}$

= **1**

So, from a dose of 1 Gray, the damage produced by direct ionization is:

1 altered site in one of the 20,000 copies of this protein

Official Système Internationale (S.I.) Prefixes

http://www.simetric.co.uk/siprefix.htm

Note: *googolplex* not listed!

Assessment of relative contributions of Direct Ionization and OH Radicals in Cells

Cell targets react with $^{\bullet}OH$;

$RH + {}^{\bullet}OH \rightarrow$ (1)

If a compound is added which competes with the target for $^{\bullet}OH$, the amount of damage to the target will be reduced:

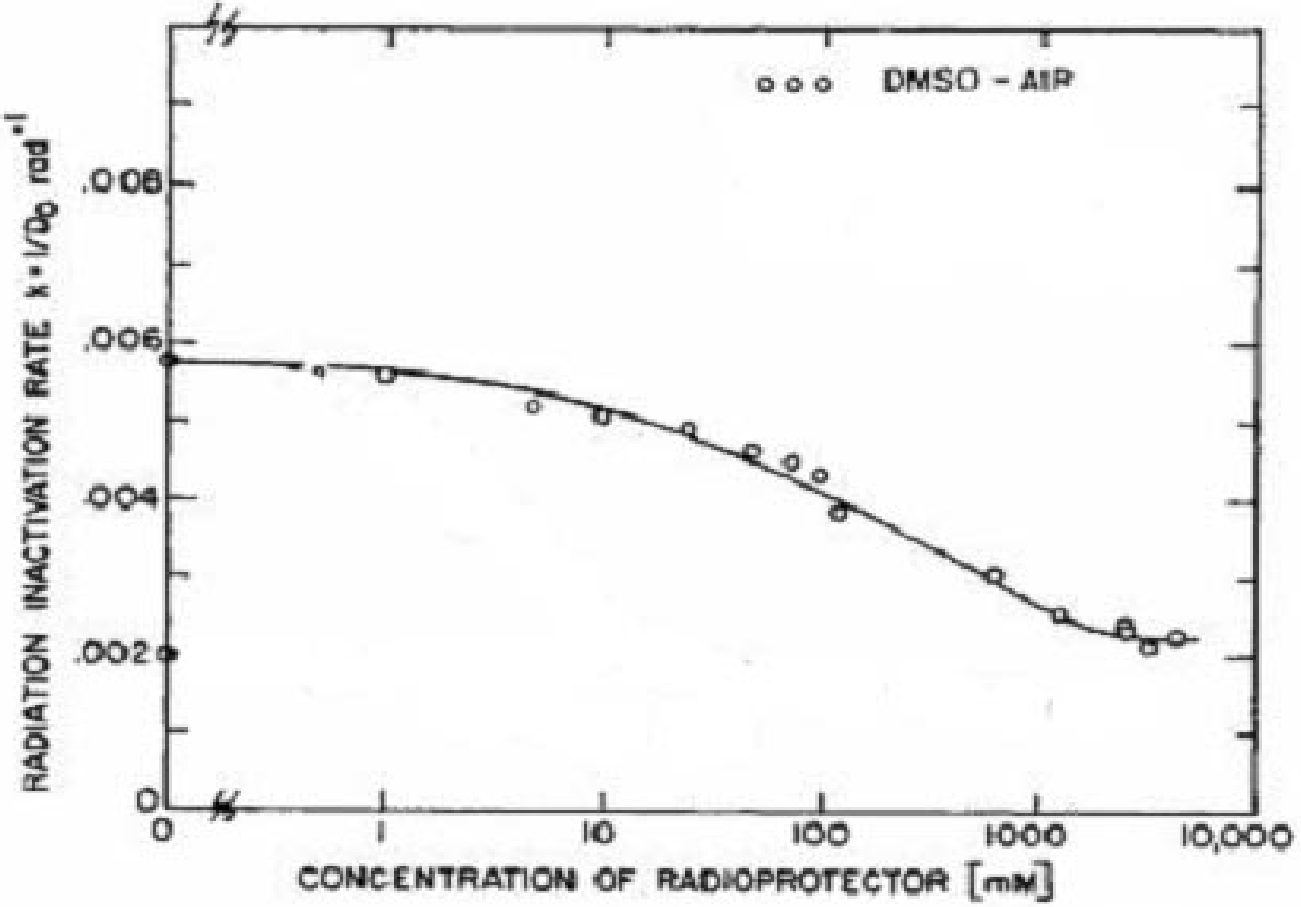
$DMSO + {}^{\bullet}OH \rightarrow$ (2)

Fraction of $^{\bullet}OH$s reacting with RH:

$$\frac{k_1[RH][{}^{\bullet}OH]}{k_1[RH][{}^{\bullet}OH] + k_2[DMSO][{}^{\bullet}OH]}.$$

Effect of DMSO on Chinese Hamster Cell Survival

*J.D. Chapman et al. Radiat. Res. **56**; 291-306.*



Effect of DMSO on chromosome aberration yield

*Littlefield L.G. et al. Int. J. Radiat. Biol. **53**; 875-890.*

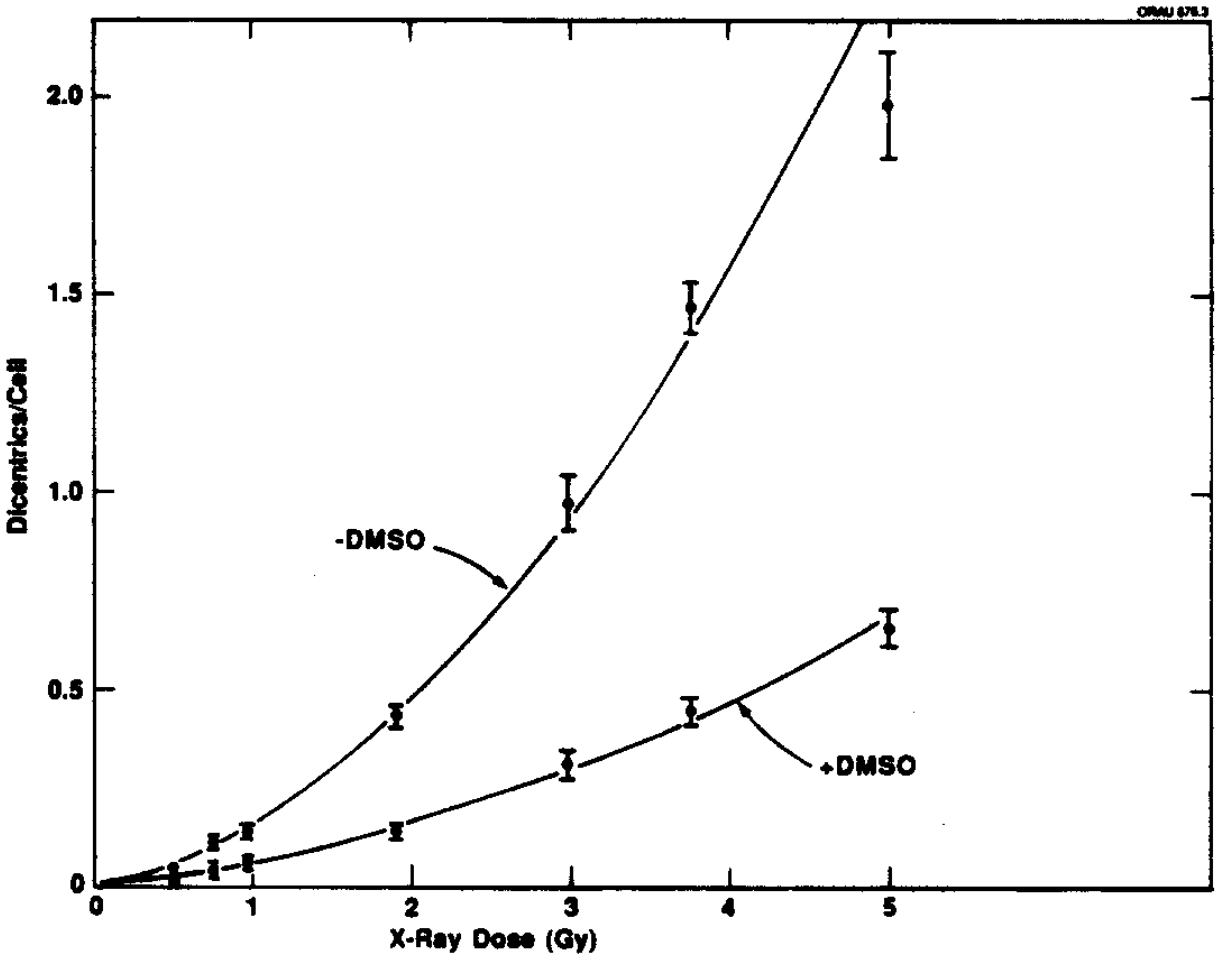


Figure 2. Dose–response relationships for dicentric induction in lymphocytes exposed to X-radiation in absence or presence of DMSO. Dicentric cell^{-1} data fitted to linear-quadratic dose–response function.

Hydroxyl Radical Scavengers Reduce Biologically Significant Damage

1964	Bacteria	*Johansen, I. and Howard-Flanders, P., Radiat. Res. 24: 184.*
1972	Mammalian Cells	*Roots, R. and Okada, S., Int. J. Radiat. Biol., 21; 329.*
1973		*Chapman, J.D. et al., Radiat. Res. 56; 291.*
1979	High LET	*Chapman, J.D., Radiat. Environ. Biophys. 16; 29.*
1984	Transformation	*Yang T.C. and Tobias, C.A., Adv. Space Res. 4; 207.*
1987	Mutations	*Corn B.W. et al., Radiat. Res. 109; 100.*
1988	Chromosome Aberrations	*Littlefield, L.G., Int. J. Radiat. Biol. 53; 875.*
1995	DNA double strand breaks – α particles	*deLara, C.M. et.al., Radiat. Res. 144; 43.*
2000	Cell killing from ^{125}I decays	*Walicka, M.A. Radiat. Res. 154; 326.*
2001	Genomic Instability	*Limoli, C.L. et al. Free Radic Biol Med.31; 10.*

Conclusions of authors:

65 % of most biological effects are caused by •OH radicals

½ maximum protection is provided by 0.13M DMSO

•OH radicals cause base damage and strand breaks in DNA.

Other evidence suggests the unimportance of OH Radicals

- Treatment with H_2O_2 does not kill cells

  (*Ward, Blakely and Joner, Radiat Res.103:383-92)*.

- High endogenous levels of oxidized sites are present in intracellular DNA (equivalent to that from ≈ 2.4 - 48 Gy).

  Collins et al. Arch Biochem Biophys. 423 57-65

- α-particles produce a lower yield of •OHs than γ-rays, but are more effective in killing cells (*Objection raised by T. Alper*).

Hydrogen Peroxide causes Hydroxyl radical damage

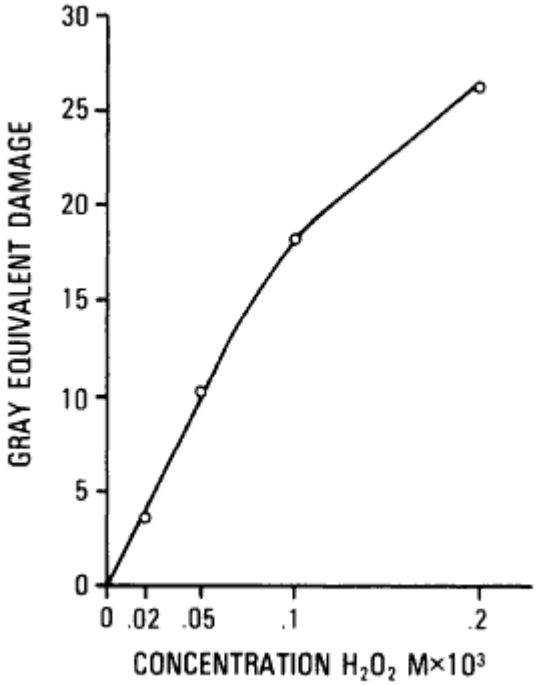
$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$

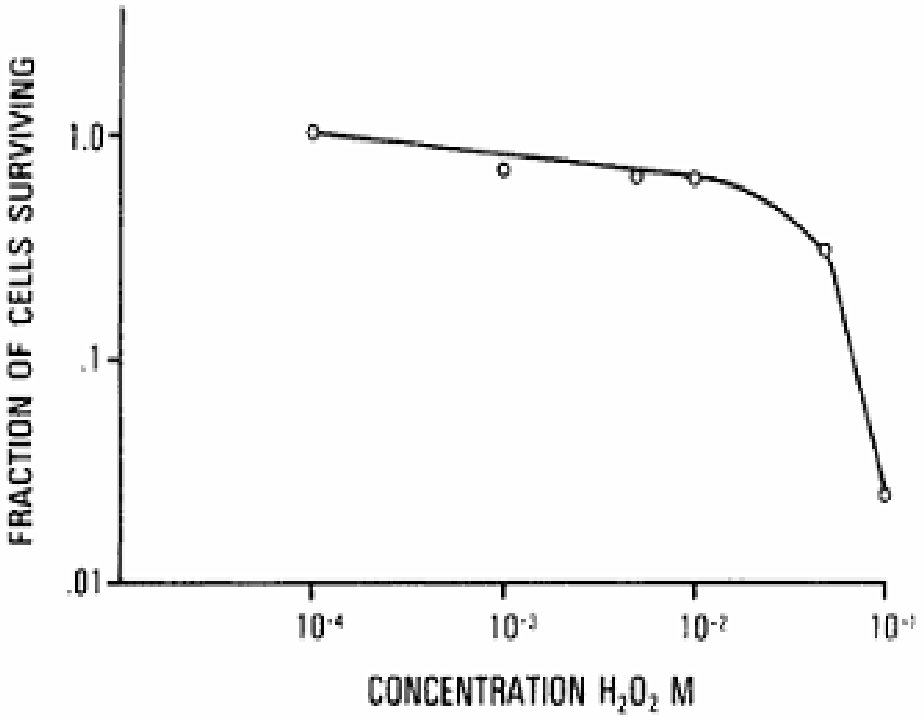
(Fenton's reagent, Haber and Weiss suggested the reaction)

The Fe^{2+} is assumed to be serendipitously bound to DNA!

Single Strand Breaks and Cell Killing by Hydrogen Peroxide.

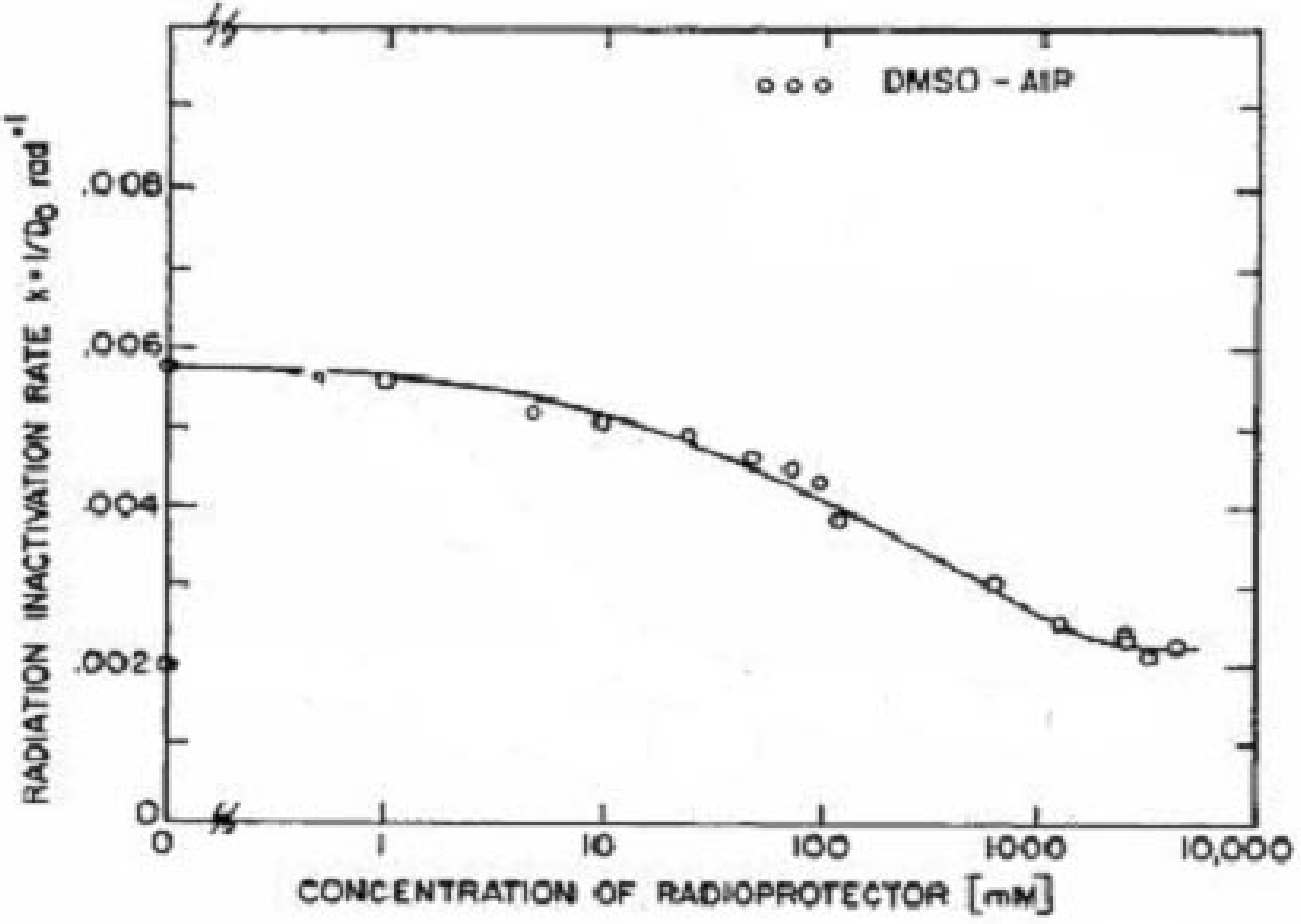
Ward, Blakely and Joner, Radiation Res. 103, 383





The scavenger data tell us more!

e.g. those of J.D. Chapman *et al. Radiat. Res.* ***56****; 291-306.*



Life Time of OH radicals in mammalian cells

Fraction of $^{\bullet}OH$s reacting with target $= \dfrac{k_1[RH][^{\bullet}OH]}{k_1[RH][^{\bullet}OH] + k_2[DMSO][^{\bullet}OH]}$.

When the amount of scavengeable damage is reduced by a factor of 2,

$$k_1[RH][^{\bullet}OH] = k_2[DMSO][^{\bullet}OH],$$

Thus, the half life of the $^{\bullet}OH$ radical in its reactions with DNA intracellularly can be determined:

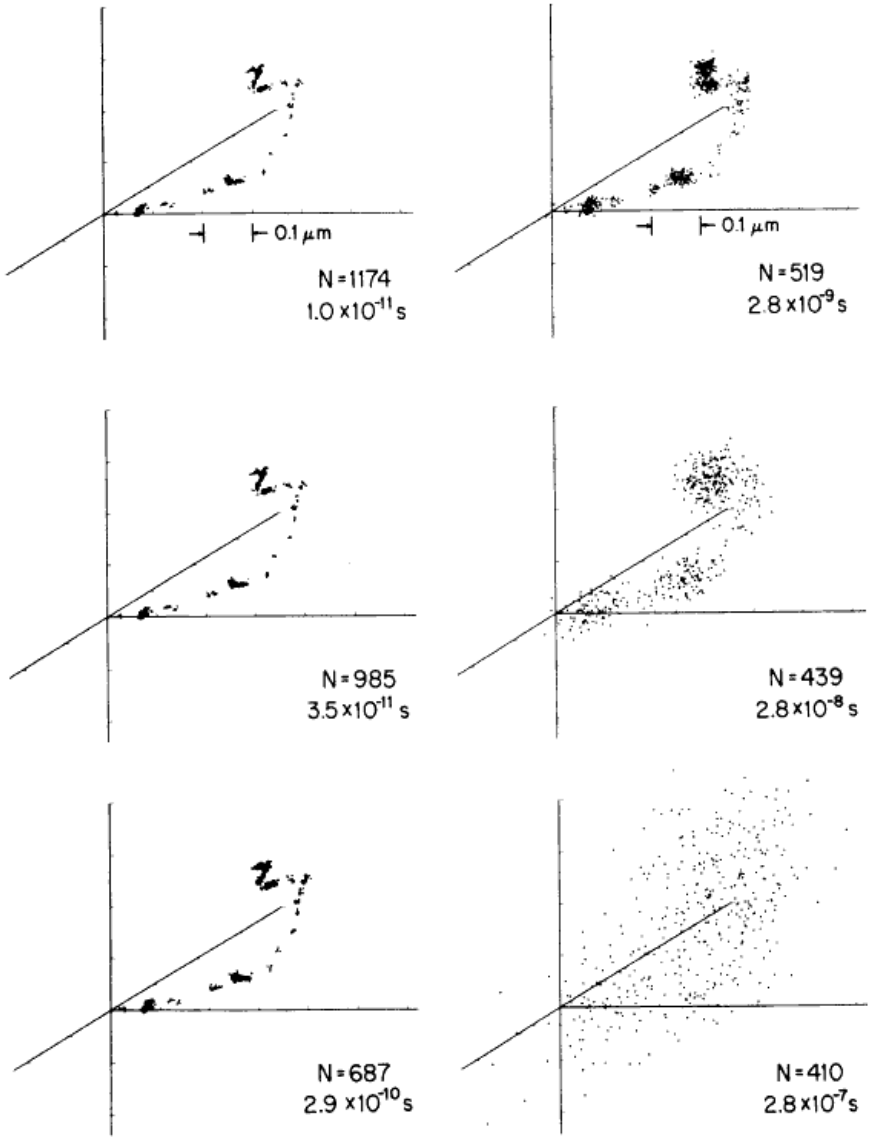
$$t_{½} = \ln 2 / k_2[DMSO]$$

So that if $[DMSO]_{½} = 0.13$ M and $k_2 = 6.5 \times 10^9\ LM^{-1}s^{-1}$

$$t_{½} = \mathbf{8.2 \times 10^{-10}\ s}$$

Evolution of an electron track

J.E. Turner et al. Radiat. Res. 96; 437.



Progression of reactive intermediates formed after deposition of radiation energy.

Changes in location and numbers as a function of time.

Dots: reactive species

N: Number present

Cellular scavenging ~ 8×10^{-10} s

Distance Hydroxyl radicals move before reacting in mammalian cells

The diffusion of a particle in 3 dimensions is $2.45\ [Dt]^{0.5}$ (*ref 1*)

For $^{\bullet}OH$, D, the diffusion constant $= 2 \times 10^{-5}\ cm^2\ s^{-1}$ *(ref 2)*

Mean distance $^{\bullet}OH$ moves in mammalian cell before reacting with DNA is:

$$2.45\ [2 \times 10^{-5} \times 8.2 \times 10^{-10}]^{0.5}\ cm$$

$$= \mathbf{\sim 3\ nm.}$$

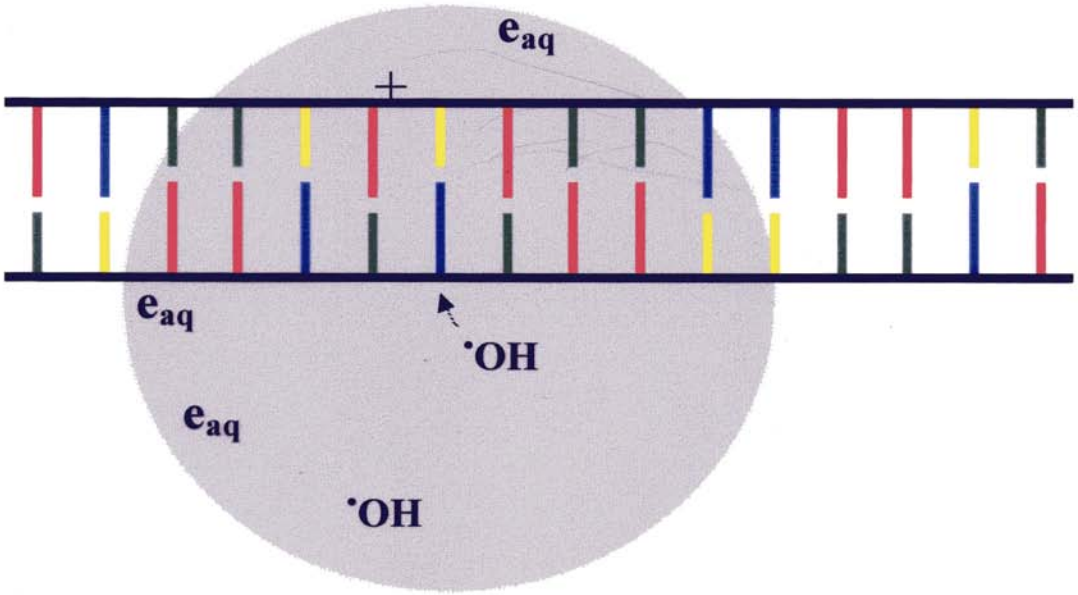
1. A. Einstein, Investigations on the Theory of Brownian Movement, ed. R. Fürth, translated by A.D. Cowper (1926, reprinted 1956); Einstein, Collected Papers, vol. 2, 170-82, 206-22

2. J.E.Turner Atoms, Radiation, and Radiation Protection, 2nd ed. New York: Wiley-Interscience, 1995. Table 13.2)

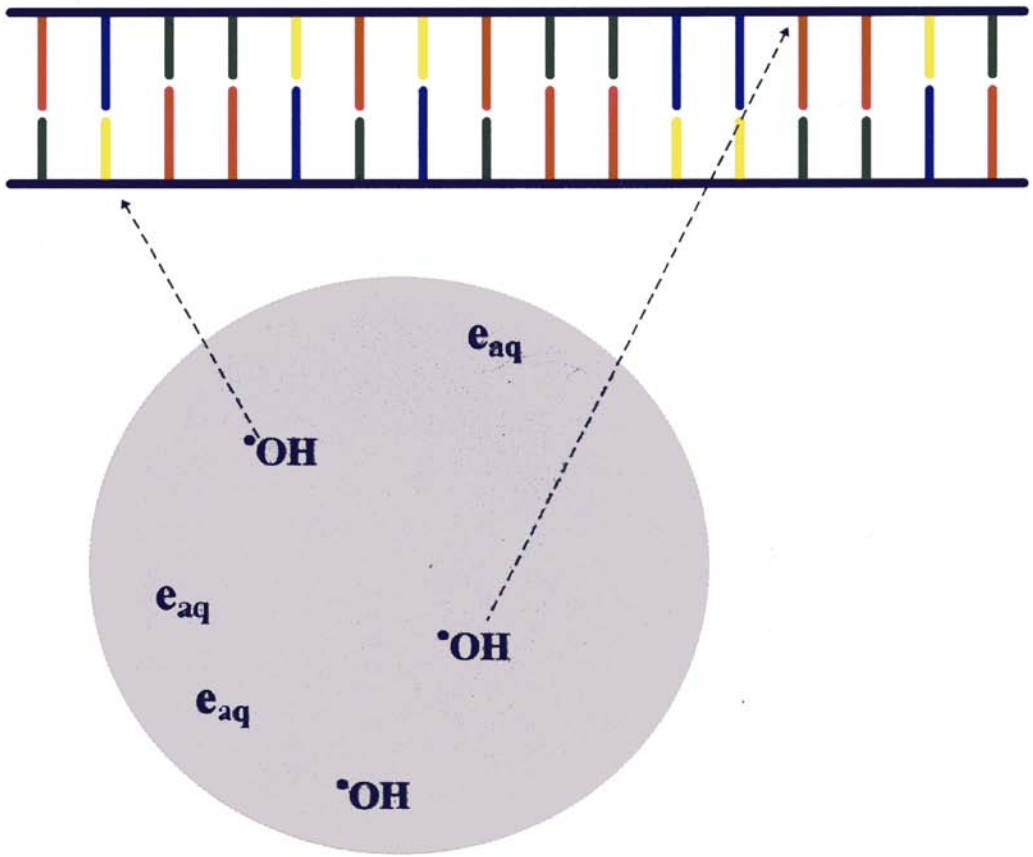
Radiation Chemical Concepts of early distributions of radicals

Entity	Energy	Size	Energy (%)	Events (%)
Spur	<100eV	4nm (diam.)	80	**95**
Blob	<500eV	7nm (diam.)	20	**5**
Short tracks	500-5,000eV			
DNA		2mm (diam)		
nucleosome		5.7nm thick		

Average Energy Deposition Event and DNA

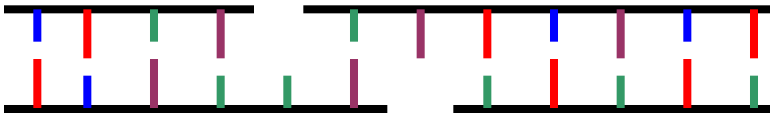


Diffusion of Radicals to DNA

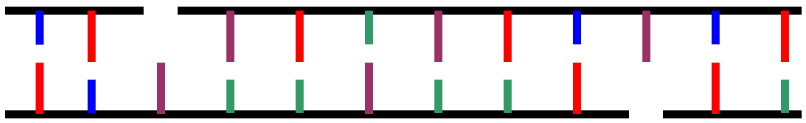


Examples of Multiply damaged sites

DSB closely opposed

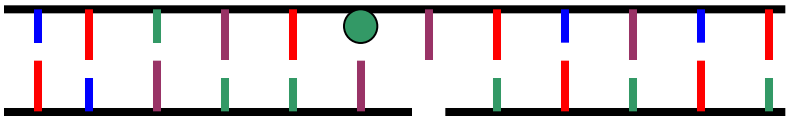


DSB separated

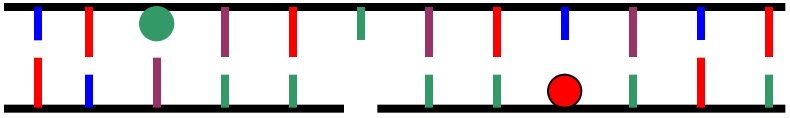


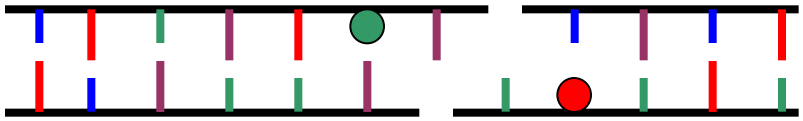
Base damages separate

Base damage and SB close



More Complex Damage





Locally
Multiply
Damage
Sites

C*ombination*
L*esions*
U*nderlying*
S*ingle*
T*rack*
E*vent*
R*adiation*
S*ignatures*

Relative LMDS Frequencies in DNA in Solution

J.R. Milligan et al. Int. J. Radiat. Biol. 76 1475-1483

Locally Multiply Damaged Site	Percent total LMDS
DSB	20
Oxy-purine complex	33
Oxy-pyrimidine complex	46

Enzymes used to cut at base damaged sites, formamidopyrimidine glycosylase (oxy-purine) and endonuclease III (oxypyrimidine) can be inhibited by neighboring damage. Therefore the yields of base damaged sites are probably higher.

Relative LMDS Frequencies in Human Cells

B. Sutherland et al. Radiat. Res. 157, 611–616

Locally Multiply Damaged Site	% Total
DSB	27.5
Oxy-purine complex	27.8
Oxy-pyrimidine complex	24.7
Abasic site complex	20

Note: Values are approximate since there are cross-sensitivities to the enzymes, and, cutting by the enzymes is inhibited by proximal damage (e.g. Weinfeld M. et al. Radiat Res. 156 584-9)

Variables of Multiply Damaged Sites

Size - distance over which damage spread

Complexity – numbers of damages per site

Composition – variety of base damages and SSB

DSB production by alternate method

Limoli and Ward Radiat. Res. 134 160-9.

DNA labeled with 5-bromouracil.

DNA loaded with Hoechst dye 33258.

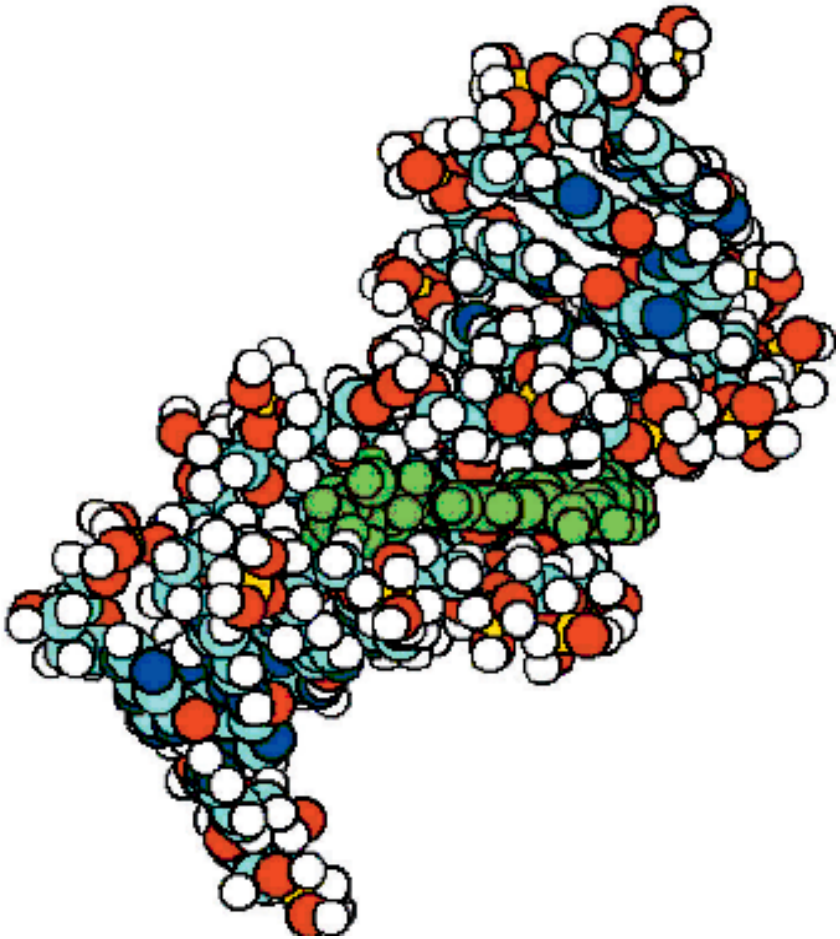
Exposed to UVA light; 360 nm (3.4 eV).

Measure DSBs and cell killing.

Hoechst 33258 binding to DNA

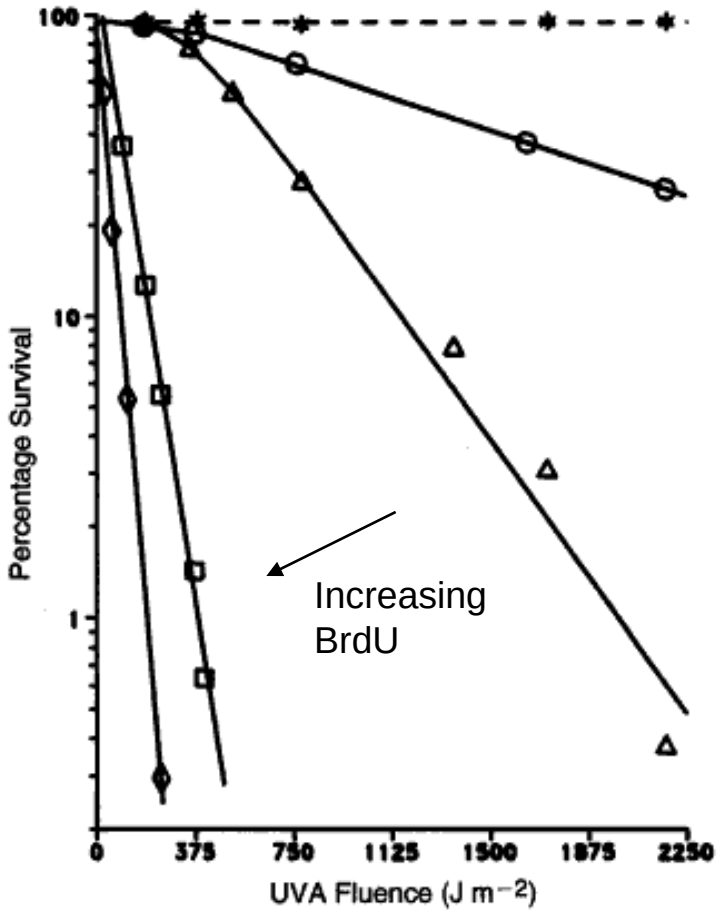
Kakkar et al. J. Biomolec. Struct. & Dynam. 23, 37

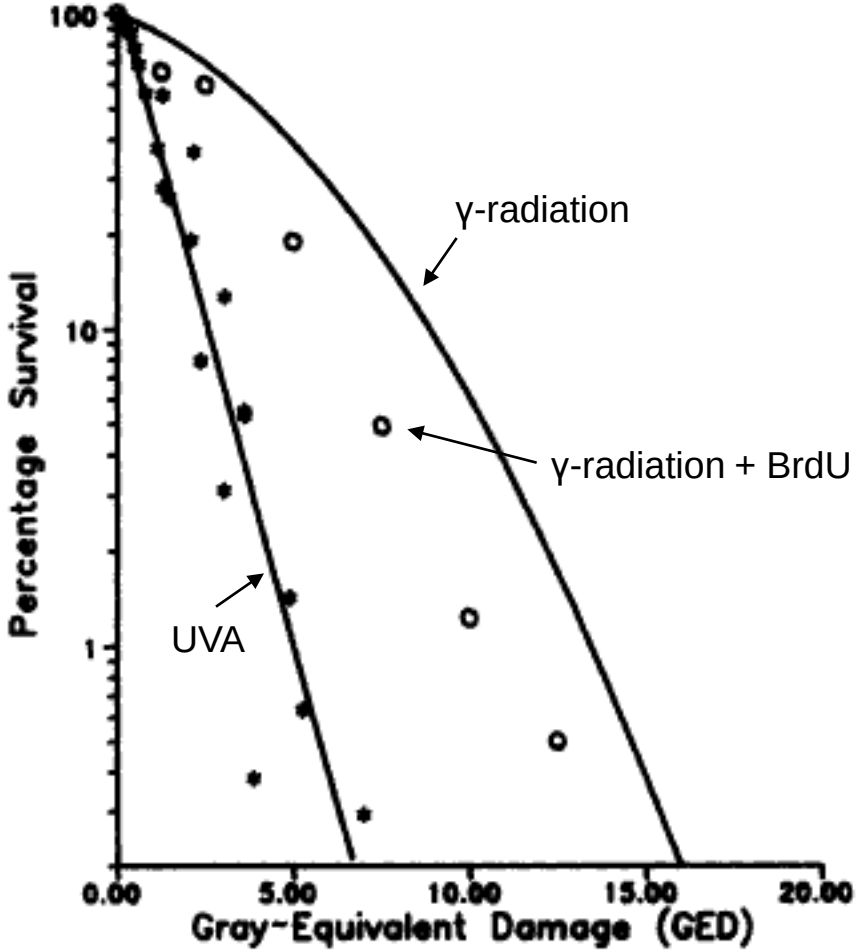
The Hoechst 33258-DNA complex. The drug molecule is shown highlighted in green.



DSB produced by photolysis (Dye, BrdU, UVA)

Limoli and Ward Radiat Res. 138 312.



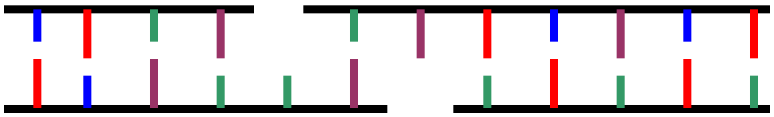


Conclusions from photolysis experiment

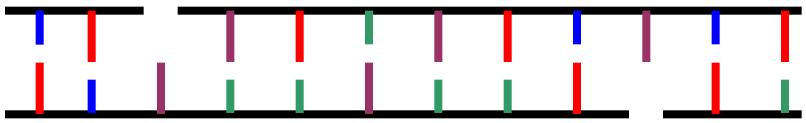
- DSBs can be produced by agents other than ionizing radiation.
- Not all DSBs are equally effective in killing cells.
- DSBs which are more closely opposed are more lethal.
- Some ionizing radiation induced DSBs are non-lethal.

Examples of Multiply damaged sites

DSB closely opposed

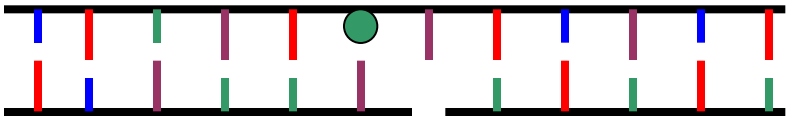


DSB separated

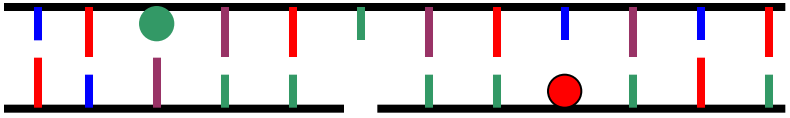


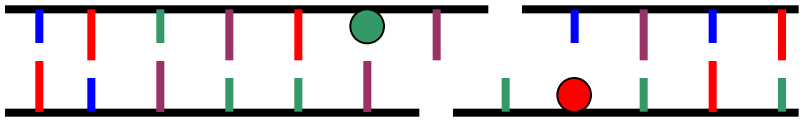
Base damages separate

Base damage and SB close



More Complex Damage





Potential Consequences of DSBs

A. Repair — Base sequence unchanged

B. Rejoining — Base sequence incorrect

C. Joining/Misrepair — Deletion

D. Non-rejoining — Deletion

DSB and mutations

HPRT gene in hamsters is 36 thousand base pairs (kbp) in length

The coding region of 648 bp are in 9 exons

Double strand break in an exon can lead to a point mutation.

Yields of HPRT point mutations and DSBs

Yield of point mutations = **8 per 10^6 cells per 2 Gy.**

(T. Morgan et al. Mutat Res. 232 171)

Size of exon 648 base pairs

Total DNA in exons in 10^6 cells = 6.48×10^8 base pairs

Yield of DSB is 5.8×10^{-3} per Gy per 10^6 base pair

(M. Löbrich et al. P.N.A.S. 92 12050)

2 Gy causes $5.8 \times 10^{-3} \times 6.48 \times 10^2$ DSB in target exons

= 8 DSB in target exons

Do all DSBs in exons yield point mutations?

The yield of mutation is equal to the yield of DSBs. But DSBs with distant SSBs would be expected to be accurately repaired.

Other damage, i.e., LMDS in which base damage is present, are present in several fold higher in yield.

Repair and rejoining of this latter kind of damage could also lead to a point mutations.

How do we measure DSBs?

a. Measurements of DSBs are carried out after stripping away all other cellular material.

Such material (e.g. nucleosomes) may act to hold the ends of DSBs in register enabling their rejoining.

b. Removing DNA from cell may also break hydrogen bonded base pairs in between SSBs on opposite strands.

The hydrogen bonding could serve to hold the ends together favoring fast rejoining.

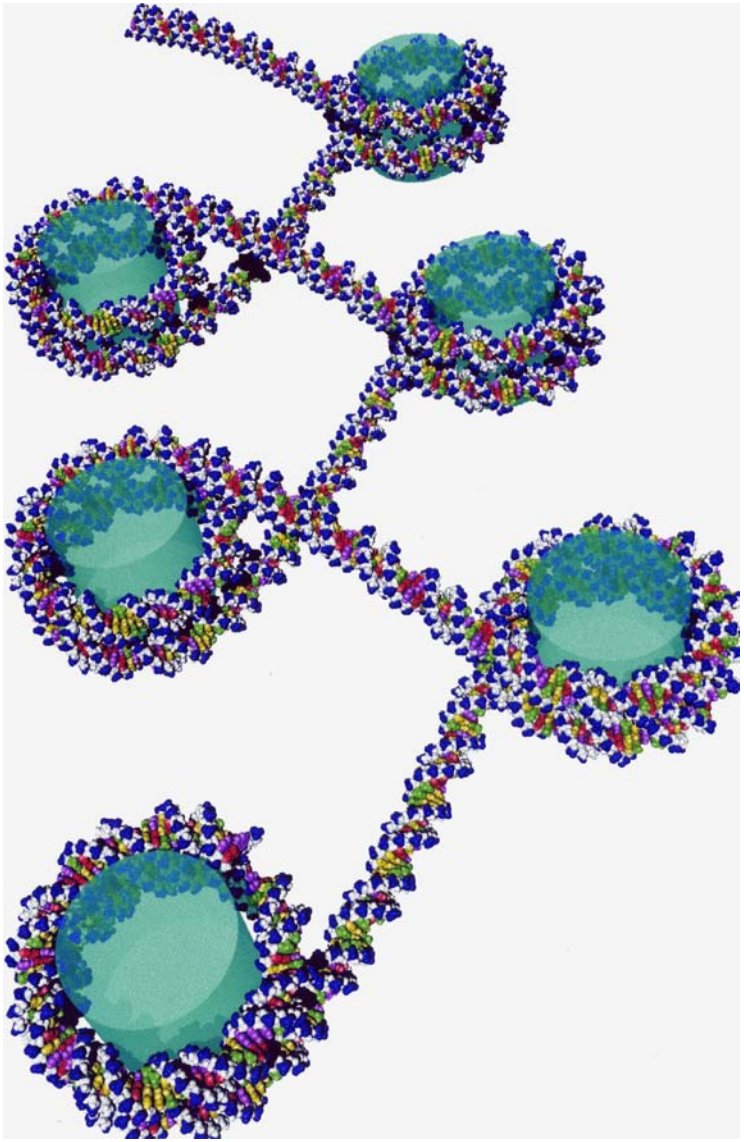
c. DSBs measured by these means are greater than the yields existing in cells.

d. Yields measured by biochemical methods (Pulse field gel electrophoresis, Elution, Centrifugation, etc.) are 37 per cell, but *in situ* by premature chromosome condensation (PCC) are 4-6 per cell.

Cornforth M. p. 563 in "DNA Damage and Repair" (ed. Nickoloff and Hoekstra) Humana Press.

DNA and nucleosomes

Friedland, W. et al.Rad. Res 150 170-182



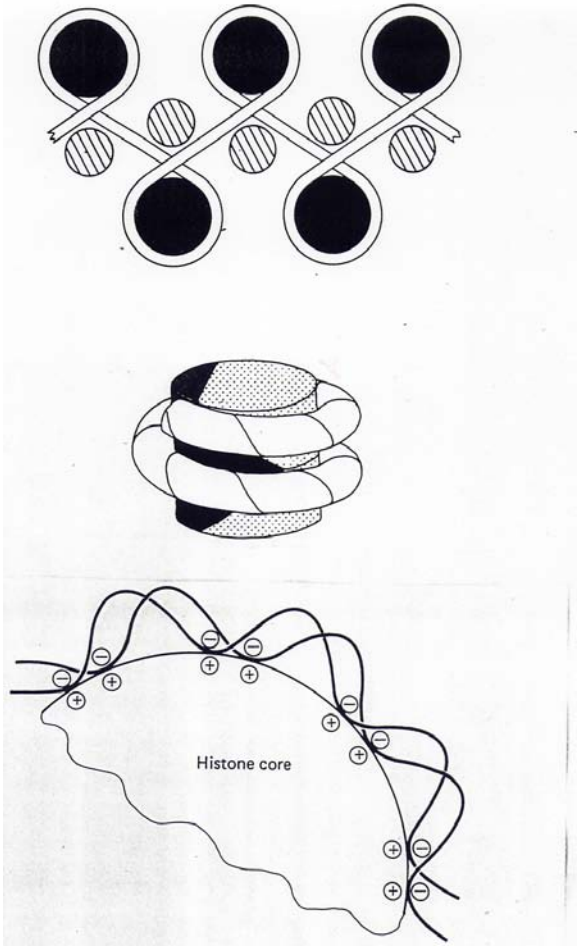
Chromatin fiber with zigzag structure.
Blue: phosphate groups; white: sugar group atoms; green, yellow, red, violet; base atoms of adenine, guanine, cytosine and thymine, respectively; turqoise; histones

The attraction between DNA (polyanion) and the multiple positive charges on the histones can serve to keep the two ends of a DSB in register, aiding correct rejoining.

Breaks occurring in the linker region may not be so stabilized and may be more prone to separate from their partner end.

Nucleohistone packaging

From: *K.E. van Holde, Chromatin, Springer Verlag.*



Three types of DSB

1. In the linker region – the ends readily separate.

2. Held together by holding the ends in register on histones.

3. Held together by hydrogen bonding between complementary bases.

Biophysical DSB measurement techniques detect all three types.

Within the cell 1,2, and 3 can have different outcomes.

DSB rejoining

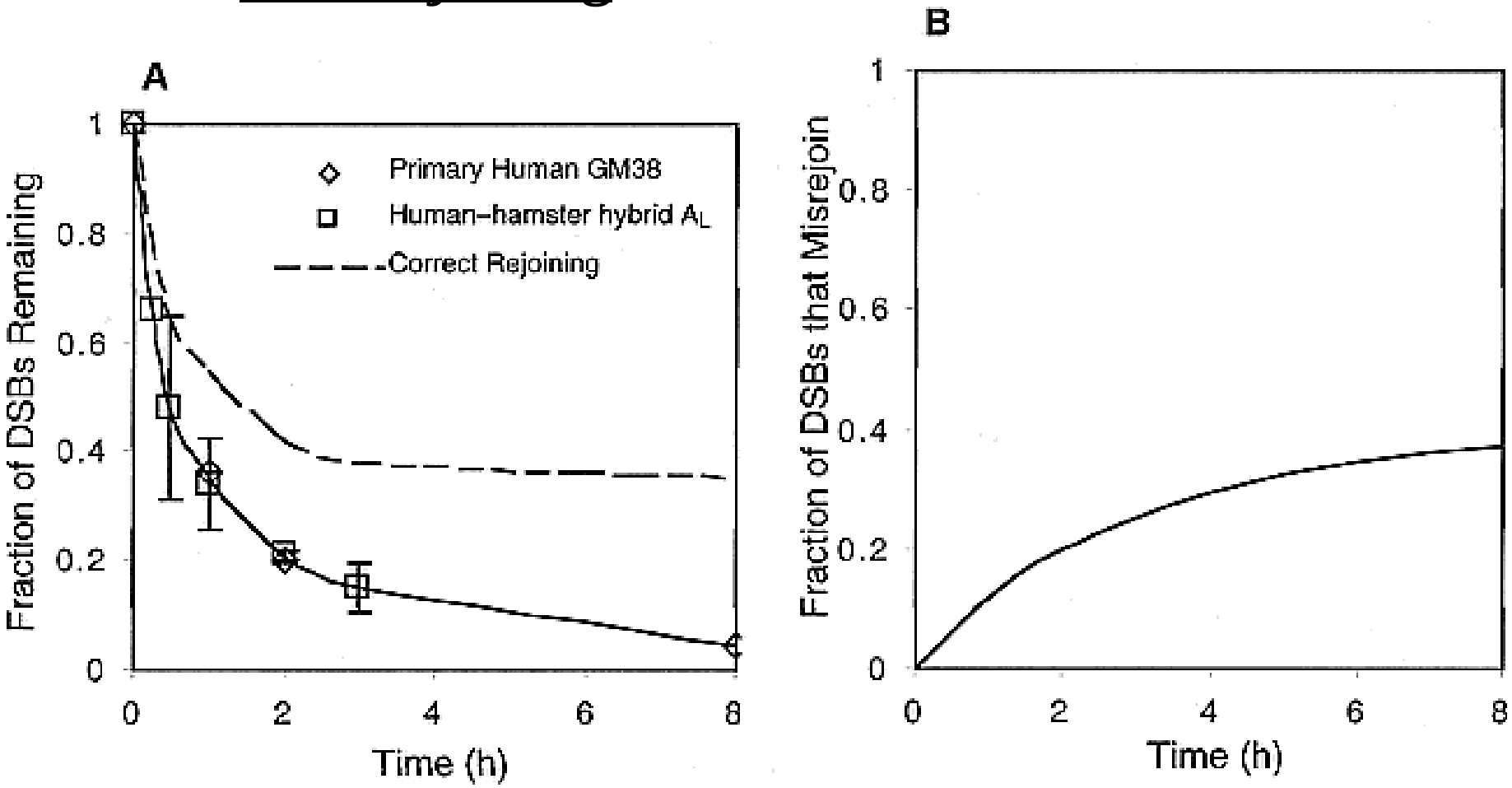


FIG. 4. Total rejoining of radiation-induced DSBs after 80 Gy X irradiation. DSB rejoining in G_1-phase A_L cells and G_0 GM38 cells (panel A) was determined by employing a standard pulsed-field gel electrophoresis assay (*38*) in which the fraction of DNA released from the well is used as an indicator of the relative number of DSBs present in the sample.

Fouladi et al. Radiat. Res. 150, 619-26.

What has been learned from in *vivo* chemistry

a. OH Radicals have short lives and travel short distances.

a. OH Radicals react close to where they are produced.

a. The clusters of ionization from radiation give rise to multiply damaged sites.

a. There is a variety of types of MDS.

a. All DSBs are not alike.

Damage produced by 1 Gray

1,000 single strand breaks

3,000 damaged bases

37 double strand breaks (measured by harsh techniques)

5 actual double strand breaks (mild techniques)

190 multiply damaged sites (harsh techniques)

In contrast, UV damage

In skin cells, **sun** exposure at an altitude of 600m. produces thymine dimers in yield equivalent to 14 Jm^{-2} s^{-1} of 253.7 nm light

Klocker et al., Eur. J. Biochem 142, 313.

This corresponds to a thymine dimer yield of

1.2×10^6 per cell per hr.

Ward, Radiat. Res. 152 104.

Cell Killing: Number of DNA lesions present at 37% Survival

Agent	DNA lesion	Number
Ionizing Radiation	SSB	1,000
	DSB	40
	DPC	440
Bleomycin	SSB	150
	DSB	30
UV light	<T-T> dimer	400,000
Hydrogen Peroxide (OH)	SSB	2,600,000
Aflatoxin	Adduct	10,000
1-Nitropyrene	Adduct	400,000
Benz[α] pyrene 4,5-oxide	Adduct	100,000
2-(N-Acetoxy-N-acetyl) amino fluorine	Adduct	700,000
Methylnitrosourea	Guanine alterations	~1,000,000

J.F.Ward, Progress in Nucleic Acid Research and Molecular Biology 35, p. 113

Some Numbers

In a cell, a dose rate of 1mGray per year

produces 1 Actual DSB (PCC) every 185 years

1 DSB every 25 years

1 SSB per year

1 base damage every 4 months

1 LMDS every 2.5 years

Human body has 10^{14} cells, a dose rate of 1mGray per year

produces <u>in 1 second</u>

2.5×10^6 Actual DSB

1.9×10^7 DSB

4.8×10^8 SSB

1.4×10^9 base damage

1.9×10^8 LMDS

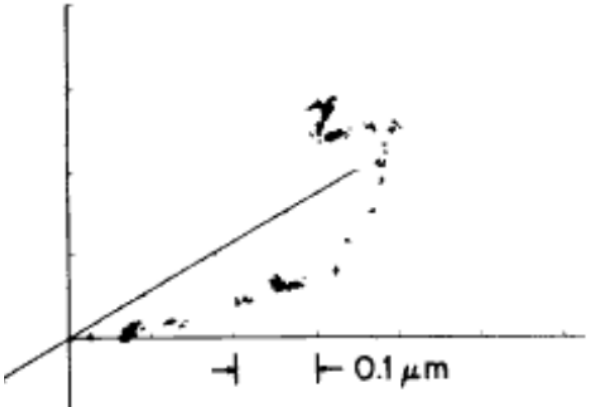
[*Abkowitz et al. Blood 100, 2665*

Number of pluripotent hematopoietic stem cells per human ~1.12×10^4 - 2.24×10^4]

? Calculate # of DSBs per dose.

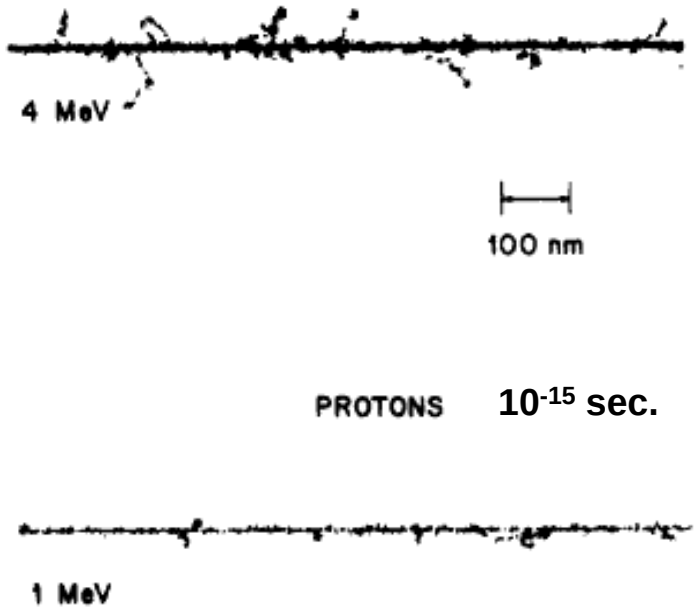
Comparison of radical distribution from alphas and protons with that of electrons

ELECTRONS 10^{-11} sec.



J.E. Turner et al. Radiat. Res. 96, 437

ALPHA PARTICLES 10^{-15} sec.



PROTONS 10^{-15} sec.

Hamm et al. Radiat. Res. 104, Suppl. 8, s20.

Other Paradigms of Radiation Action

Apoptosis

Bystander Effect

Chromosome Instability

Death Inducing Effect

Gene Induction

Low Dose Hypersensitivity

Protein Mobilization

From: **The Breakdown of Desoxyribonucleic acid under Deuteron and Electron Bombardment.**

C.L. Smith Arch Biochem. Biophys. 46. 12-17

Equivocation

"The assumption made is plausible and perhaps not too improbable

and is possibly true in essence if not in detail. It has not, however, yet

been confirmed by experiment."